The Effect of Subcutaneous Naloxone on Experimentally-Induced Pain

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ABSTRACT

The heat pain threshold was assessed in 32 healthy participants after a mild burn on the dorsal surface of each hand, after injection of an opioid antagonist (80 μg naloxone) or vehicle alone (0.2 mL saline) into the burnt skin of one hand, and after repeated painful immersion of this hand in cold water for up to 180 s. We hypothesized that sensitivity to heat would decrease at the burn-injured site after the immersions, due to local release of opioids into the burnt skin. Naloxone augmented cold-induced pain during the immersions in participants who tolerated the longest immersions, implying that release of endogenous opioids suppressed cold-pain. After the immersions, sensitivity to heat decreased at the burn-injured site in the immersed hand, but naloxone did not block this effect. Instead, naloxone altered sensitivity to heat in unburnt skin, implying that thermal hyperalgesia at sites of burn injury masked the modulatory effects of opioids. In particular, naloxone blocked a decrease in sensitivity to heat at an unburnt site on the contralateral hand of participants who tolerated the longest immersions, consistent with central or systemic opioid release. Naloxone *reduced* sensitivity to heat at unburnt sites in participants who tolerated medium-length immersions, suggesting that an increase in systemic or central opioid activity evoked thermal hyperalgesia in this group. In addition, in a small group of participants who tolerated only brief immersions, naloxone blocked decreases in sensitivity to heat at an unburnt site in the immersed hand. These findings suggest that repeated painful immersions trigger local opioid release in participants who tolerate only brief immersions, and elicit central or systemic opioid release in participants who tolerate longer immersions.

Perspective: This article demonstrates that repeated immersion of the hand in painfully cold water increases opioid activity, and that the increase in opioid activity exerts multiple opposing effects on sensitivity to heat. Individual differences in the response to opioids might contribute to individual differences in pain tolerance.

Introduction

Endogenous opioids, released within the central nervous system and from the adreno-hypophyseal axis, inhibit pain during stress.[24,39] This stress-induced analgesia may have evolved to increase the likelihood of survival during times of physical threat, thereby facilitating self-defense and avoidance of further injury.[1,38] In rodents, stressful procedures such as electric shocks and cold-water swims can evoke central opioid-mediated analgesia.[34,35] In humans, the opiate receptor antagonist naloxone has been shown to block analgesia following cognitive stress,[3] anticipation of painful foot shock,[41-43] and the immersion of one foot in ice-water.[20] Naloxone also augmented thermal hyperalgesia induced by the topical application of capsaicin.[2] Although such findings indicate that endogenous opioids contribute to analgesia, the site of action is uncertain because the dose of naloxone used in these studies was sufficient to produce substantial blockade of opiate receptors both within and outside the central nervous system.

A component of endogenous opioid analgesia may be mediated peripherally. In rats, the withdrawal threshold to pressure applied to an inflamed paw increased after an ice-water swim, due to local release of β-endorphin from immune cells.[26,27,31] In humans, synovial fluid obtained during knee surgery contained immune cells, many holding β-endorphin.[33] The intra-articular injection of naloxone immediately following knee surgery increased pain ratings, scores on the McGill pain questionnaire and consumption of analgesics,[33] consistent with peripheral opioid analgesia.

We recently found evidence of peripheral opioid analgesia in an experimental burn model in humans.[29] In this model, naloxone (80 μg) was injected subcutaneously at a site of mild burn injury in the forearm of 24 volunteers, and saline was injected at another burn site in the other forearm. The naloxone pre-treatment blocked the local antihyperalgesic effect of 10 μg fentanyl but did not block the antihyperalgesic effect of fentanyl injected contralaterally,

consistent with local opioid receptor blockade.

The primary aim of the present study was to investigate the effect of repeatedly immersing one hand in cold water on heat-pain sensitivity at sites of burn injury (intended to evoke an inflammatory response that facilitated peripheral opioid analgesia) and elsewhere in both hands. Analgesia develops following the repeated immersion of the hand in cold water,[37] but the mechanism of this analgesia is unknown. The pain and distress evoked by the cold-water immersions might activate opioid-sensitive pain modulation processes in the central nervous system.[3,20,34,35,41-43] In addition, cold-water immersions could accelerate the local release of endogenous opioids from immune cells.[26-28,30-33] If so, this should reduce sensitivity to noxious stimulation in the immersed hand relative to the contralateral hand. Moreover, a higher concentration of immune cells at the site of burn injury might augment local opioid analgesia. To investigate this possibility, 80 μg naloxone or saline was injected subcutaneously at the burn-injured site in the immersed hand.

A second aim was to investigate effects of opioid blockade on pain tolerance during the cold water immersions. Naloxone augments shock-induced pain and cortical evoked potentials in pain-insensitive individuals, but reduces pain and evoked potentials in pain-sensitive people.[6] Thus, we expected that the hyperalgesic effects of opioid blockade would be greatest in pain-tolerant participants**.**

Method

Participants

The sample consisted of 17 men and 15 women aged between 17 and 39 years (median age 19 years). They were informed that the experiment aimed to investigate the effect of the opiate antagonist naloxone on pain sensitivity induced by a mild burn and repeated immersion of their hand in cold water. Each participant received A$20 for time spent and provided informed consent for the procedures, which were approved by the Ethics Committee of the

University of Western Australia.

Apparatus and Procedures

The sequence of procedures is summarized in Table 1. Participants attended the laboratory on two occasions, separated by 4 to 7 days. The temperature of the laboratory ranged between 20 and 23ºC. On each occasion, the 10-mm diameter probe of a thermocouple-controlled cautery unit, heated to 48^{o}C, was applied with a force of approximately 1 N in hairy skin over the first dorsal interosseous muscle of each hand for 120 s to produce a mild burn.[11] Thirty minutes later, heat-pain thresholds (HPT) were measured at the burn site and at a control site 2-5 cm laterally from each burn site on both hands. Although the burn sites were approximately the same on each occasion, the HPT after the burn did not differ systematically from the first to the second occasion, irrespective of whether naloxone or saline was injected at the site of burn injury on the first occasion. Thus, effects of the burn and injection on the HPT appeared to have dissipated by the second occasion.

The HPT was determined by directing radiant heat from a halogen globe through a lens and circular aperture (1.1 cm in diameter) onto the skin. Skin temperature was measured by a thermistor which was positioned under an aluminum shield in the centre of the aperture. The arm of the lamp was adjusted to allow the thermistor to touch the skin lightly without transferring the weight of the lamp. Skin temperature was held at a baseline of 35.6ºC for 10 s and then increased linearly at 0.5ºC per second. Participants switched the heat lamp off when they first felt pain (the HPT) or the heat lamp was automatically switched off when the temperature reached 47^{o}C. Two HPT estimates were conducted at each site, with a third administered if the first two differed by more than 2ºC. Participants wore an eye-mask during sensory testing to minimize visual cues that might otherwise have affected the HPT.

After the post-burn assessment, 0.2 mL 0.9% saline, with or without 80 μg naloxone hydrochloride, was injected subcutaneously into one of the burn sites (the dominant hand on

50% of occasions). The injection was administered double-blind, and half the participants received naloxone in the first session. The HPT was measured again starting five minutes after the injection.

Skin temperature was then measured with an insulated thermistor at the four test sites, and participants began a series of immersions of the hand that had been injected with naloxone or saline. On each trial, participants immersed their hand up to the wrist in stirred 2^{o}C water for as long as they could or until three minutes had elapsed. The immersion duration at pain onset and the total immersion duration (the immersion pain tolerance) were recorded for each trial. Upon removal of the hand, participants gave a verbal rating of the maximum pain experienced during the immersion, ranging from 0 (*no pain*) to 10 (*worst pain ever*). The hand remained out of the water for 20 s and was then re-immersed. Each participant completed six to ten trials. Trials were discontinued when one of the following criteria[37] was met: (1) at least six trials were completed and cumulative immersion time across the trials was at least 10 minutes; (2) at least six trials were completed and immersion time was three minutes on three consecutive trials; or (3) ten trials were completed.

Upon completion of the cold-water immersions, the immersed hand was placed in warm water at 33 $\pm$ 1ºC for 1-2 minutes and was held in front of a fan heater at the maximum comfortable temperature for a further 2-3 minutes. The cold-induced pain subsided rapidly during re-warming. Warming was discontinued when skin temperature approximated pre-immersion levels. The HPT was then re-measured two more times, with a 5-minute interval between the first and the second measure.

Data are displayed as means (*M*) $\pm$ standard error of the mean. Experimental effects were investigated in repeated measures analyses of variance and with Pearson's correlation coefficient (*r*). The multivariate solution was employed for repeated measures factors with more than two levels.

RESULTS

Changes in skin temperature

Mean skin temperature was greater at the burnt sites than the unburnt sites (*M* [burnt] = 31.3 $\pm$ .3 ^{o}C, *M* [unburnt] = 30.8 $\pm$ .3 ^{o}C; F (1,30) = 44.5, p <0.001), but did not differ between the naloxone and saline sessions, between the immersed and non-immersed hand, or from before to after the immersions (Table 2). Thus, the inflammatory response at the burn sites increased skin temperature, and warming the hand after the immersions returned skin temperature to pre-immersion levels.

Effect of naloxone on pain during the immersions

As opioid release during the immersions might influence pain sensitivity, we examined the effect of the opioid antagonist naloxone on pain during the immersions. The average duration of immersion correlated strongly across sessions, r (30) = .69, p < .001 (Figure 1A) but, nevertheless, was shorter in the naloxone session than the saline session in the most pain-tolerant participants, r (30) = .42, p < .05 (Figure 1B). To investigate this further, we allocated participants to three groups defined by their immersion duration scores in the saline session: those with a mean immersion duration per trial of less than 35 s (range 9-34 s), who were regarded as *pain-intolerant* (N = 7; lower 22% of the distribution), those with a mean immersion duration per trial of 57-128 s (*mid-range;* N = 16; middle 50% of the distribution), and those with an immersion duration of 142-180 s (*pain-tolerant;* N = 9; upper 28% of the distribution) (Figure 1B). The average duration of immersion was greater in the saline session than the naloxone session in pain-tolerant participants (168 $\pm$ 5 s versus 132 $\pm$ 16 s, t (8) = 2.89, p < .05) but did not differ between the saline and naloxone sessions in the other two groups. Sex, age, and mean HPT in burnt and unburnt skin before the immersions were similar in each of the three groups (Table 3).

The effect of naloxone on the immersion duration at pain onset, pain tolerance, and on

cold-pain ratings was investigated over the first six immersions, which were completed by all participants. The time taken to reach the pain threshold increased over the first six immersions (linear component of the Trials main effect: F (1,29) = 12.2, $p < .01$), primarily in mid-range and pain-tolerant participants (Figure 2A-C), and was greater by the sixth trial of the saline session than the naloxone session in pain-tolerant participants (main effect for Drug: F(1,29) = 5.51, $p < .05$; linear component of the Trials x Drug interaction: F (1,29) = 5.23, $p < .05$; Figure 2C). As shown in Figure 2D-F, cold-pain tolerance also increased over the first six trials in mid-range and pain-tolerant participants (linear component of the Trials main effect: F (1,29) = 7.37, $p < .05$), and was greater in the saline than the naloxone session in pain tolerant participants during Trials 2-4 (Pain Group x Drug interaction: F (2,29) = 5.21, $p < .05$; quadratic component of the Pain Group x Drug x Trials interaction: F (2,29) = 4.08, $p < .05$; Figure 2F). Pain ratings over the first six immersions are presented in Figure 2G-I. Ratings decreased during the last few immersions in pain tolerant participants (quadratic component of the Trials main effect F (1,29) = 7.16, $p < .01$; Figure 2I), and were greater during Trials 3 and 4 of the naloxone session than the saline session in this group (Pain Group x Drug interaction: F (2,29) = 4.18, $p < .05$).

These findings suggest that opioid release was greatest in pain-tolerant participants during the cold-water immersions. Thus, changes in sensitivity to heat after the immersions were explored in relation to individual differences in cold pain tolerance in analyses of variance. The results of these analyses are presented below.

Effect of the injections and the immersions on the HPT

Preliminary analyses indicated that the HPT was lower at the burnt sites than the unburnt sites 30 minutes after the burns (M [burnt] = 41.1 $\pm$ 0.35 ^{o}C; M [unburnt] = 42.5 $\pm$ 0.46 ^{o}C; t (31) = 7.04, $p < .001$), and that thermal hyperalgesia persisted at the burn sites after the cold water immersions (Figure 3A-B).

Changes in the HPT from before to after the injections were investigated in Drug (saline, naloxone) x Phase (before versus after the injections) x Injection Site (ipsilateral, contralateral) x Pain Group (pain-intolerant, mid-range, pain-tolerant) repeated-measures analyses of variance, separately for burnt and unburnt sites. None of the main effects or interactions was significant, indicating that neither the saline nor the naloxone injection had any immediate effect on the HPT and implying that opioid release did not affect sensitivity to heat before the cold-water immersions.

Changes in the HPT from before to after the immersions were investigated in Drug (saline, naloxone) x Immersion (immersed, non-immersed) x Phase (before immersions, and two measures after the immersions) x Pain Group (pain-intolerant, mid-range, pain-tolerant) repeated-measures analyses of variance. To simplify Phase effects, each measure after the immersions was compared with the pre-immersion measure in planned contrasts.

Burnt sites. The HPT increased at the burnt site in the immersed hand immediately after the immersions, but not at the burnt site in the non-immersed hand (Phase x Immersion interaction, F (2,28) = 8.57, $p < .001$; Figure 3A-B). Neither the Pain Group nor the Drug condition influenced thermal hyperalgesia at the burnt sites (none of the effects that involved these factors achieved statistical significance).

Unburnt sites. Although the Drug condition had no consistent effect on the HPT at unburnt sites (Figure 3C-D), Drug effects differed among the pain tolerance subgroups (interaction between Pain Group, Drug, Immersion and Phase (pre-immersion to second post-immersion measurement), F (2,29) = 5.27, $p < .05$). In pain-tolerant participants, naloxone *blocked* thermal hypoalgesia at the unburnt site in the non-immersed hand (Figure 4A). However, in the mid-range group, naloxone *evoked* thermal hypoalgesia at unburnt sites in both hands (Figure 4B). In pain-intolerant participants, naloxone blocked thermal hypoalgesia after the immersions only at an unburnt site in the immersed hand (Figure 4C).

DISCUSSION

The effect of the immersions on the HPT

The primary aim of this study was to determine whether subcutaneous injection of naloxone at a site of burn injury in the hand would modify thermal hyperalgesia locally after the hand was immersed repeatedly in painfully cold water. Sensitivity to heat decreased at the burnt site in the immersed hand after the immersions, but was unaffected by opioid receptor blockade.

The mechanism of this reduction in sensitivity to heat is uncertain. To minimize the possibility that cold-induced nerve conduction block would influence sensitivity to heat after the immersions, the hand was re-warmed to pre-immersion temperatures before measuring the HPT. Myelinated fibres appear to be more susceptible to cold-induced conduction block than non-myelinated fibres.[15] Washington et al.[37] reported that conduction velocity along myelinated fibres was unaltered after the hand was re-warmed following repeated cold-water immersions. Given that non-myelinated fibres contribute to heat-pain perception in humans,[17,40] an effect of cold-induced conduction block on the HPT after the hand was re-warmed seems unlikely. Moreover, the progressive decrease in sensitivity to heat in the immersed hand after the immersions is not consistent with a cold-induced nerve conduction block, which should dissipate rather than intensify over time.

Nevertheless, a local effect of the immersions appeared to contribute to hypoalgesia, because it developed sooner at the burn site in the immersed hand than in the non-immersed hand. Indeed, sensitivity to heat *increased* transiently after the immersions in the non-immersed hand. This finding was unexpected, because processes such as diffuse noxious inhibitory controls generally suppress painful sensations after intense noxious stimulation.[36] The mechanism of contralateral hyperalgesia after the immersions requires further investigation.

Individual differences in tolerance of cold-pain

Willingness to tolerate cold-evoked pain varied greatly within our sample of healthy young adults. This variation was unrelated to age, sex, or heat-pain thresholds before the immersions. Geisser and colleagues[16] found that pain-intolerant individuals reported more unhelpful coping strategies (catastrophizing, praying, and hoping), and fewer helpful coping strategies (coping self-statements and ignoring the pain) than pain-tolerant participants. In addition, pain-intolerant individuals considered that they had less control over pain and their ability to reduce it than pain-tolerant individuals. Similarly, Chen et al.[8] reported that anxiety and general fearfulness were associated with pain intensity ratings in pain-intolerant but not pain-tolerant participants. Although the generality of the pain tolerance dichotomy has been questioned,[21] individual differences in pain sensitivity and tolerance are associated with biological markers.[12,18] For example, genetic variations that regulate levels of catecholamine-*O*-methytransferase[12] may influence pain sensitivity and opioid activity in pain-tolerant people. In addition, activity in cerebral cortical regions that process painful sensations and that coordinate emotional responses to pain is greater in pain-sensitive than pain-insensitive individuals,[9] possibly due to inhibitory effects of opioid release in pain-insensitive people.[6]

In the present study, naloxone augmented cold-induced pain in the pain-tolerant group. In particular, pain tolerance was lower and pain ratings were higher in the naloxone than saline condition during the third and fourth immersion trials, and pain began sooner in the naloxone than the saline condition in the sixth trial. Why the onset of the hyperalgesic effects of naloxone differed across the different pain measures is unclear; however, it seems reasonable to assume that noxious stimulation would trigger opioid release more readily above than below the pain threshold. If so, opioid effects on the pain threshold would, by necessity, lag behind opioid effects on pain tolerance and pain ratings. In addition, psychological factors (e.g., fear of pain or low self efficacy) that provoke opioid release – and which may then be

modified by this release – might have a greater influence on pain tolerance and ratings than on pain thresholds. In any event, the findings suggest that individual differences in evoked opioid release during repeated painful stimulation contribute to individual differences in pain tolerance.

After the immersions, naloxone antagonized decreases in heat-pain sensitivity in pain-tolerant participants at an unburnt site in the non-immersed hand (i.e., at some distance from the site of injection, implying that naloxone had entered the systemic circulation). In animal studies, central opioid-mediated analgesia develops during prolonged stress,[10] particularly when the stress is inescapable.[5,19] Similarly, experimentally induced stress evokes opioid analgesia in healthy humans,[3,20,41-43] and may have induced a similar response during lengthy immersions in the present study. Alternatively, naloxone may have acted peripherally on opioid receptors in the non-immersed hand. Why this was limited to the unburnt site in the non-immersed hand is unclear; one possibility is that hyperalgesia due to the burn injury, and/or hypoalgesia after the immersions, masked the modulatory effect of opioid release.

Naloxone produced quite different effects at the unburnt site in the non-immersed hand in participants who tolerated medium-length immersions. In particular, naloxone facilitated thermal hypoalgesia, implying that naloxone decreased or opioids increased sensitivity to noxious heat. It is interesting to note the parallels between our findings and those of Buchsbaum et al.,[6] who reported that naloxone inhibited shock-induced pain in pain-sensitive individuals but enhanced shock-induced pain in pain-insensitive individuals. Low levels of opiate drugs such as morphine induce hyperalgesia;[22] thus, it is tempting to speculate that minor opioid release in the central nervous system augmented thermal hyperalgesia after medium-length immersions. Alternatively, systemic release of opioids may have indirectly sensitized peripheral nerves via inflammatory mechanisms.[13,14,23,25]

Naloxone also blocked a decrease in sensitivity to heat in pain-intolerant participants at

the unburnt site in the immersed hand after the immersions. As this was limited to the immersed hand, this finding is consistent with local opioid analgesia. In animal studies, peripheral opioid analgesia during cold-water swims is mediated by opioid receptors on the peripheral terminals of sensory nerves which are stimulated by β-endorphin.[26,28,31,32] The β-endorphin appears to be released from immune cells by corticotrophin-releasing factor,[30] interleukin-1,[7] and noradrenaline.[4]

Methodological issues

Immersion duration was controlled by the participants rather than the experimenter, and may reflect a stable individual trait that moderates sensitivity to pain. Thus, further studies that systematically vary the immersion duration are required to determine whether immersion duration or individual differences in pain tolerance affect opioid activity.

We expected that burn-induced inflammation would intensify local opioid analgesia due to an accumulation of immune cells at the site of burn injury. However, for unknown reasons, effects of naloxone were weaker at burnt than unburnt sites. One potential explanation is that local vascular or inflammatory responses during the immersions increased accessibility to peripheral opioid receptors at unburnt sites. Alternatively, the inflammatory effect of the burn might have spread to adjacent skin in the immersed hand, or the burn injury may not have been severe enough to result in local accumulation of immune cells.

Based on our previous work,[29] we expected that effects of naloxone would be greatest at the site of injection. However, the present findings suggest that naloxone acted at a distance to augment (after medium-length immersions) or block (after lengthy immersions) thermal hypoalgesia contralateral to the site of injection. Studies involving injection of naloxone in the non-immersed hand may help to further distinguish between the effects of local versus systemic or central opioid release on sensitivity to heat-pain in this experimental paradigm. The effects of systemic and local opioid release on other nociceptive modalities (e.g., pain to

pressure, cold, and punctuate stimulation) should also be explored.

Finally, investigation of the effects of naloxone in relation to cold-pain tolerance should be considered exploratory in the present study, because the sample size was small. In particular, findings in pain-intolerant subjects must be treated with caution because of the small number of participants in this group. Groups were defined *post hoc* at noticeable, but perhaps serendipitous, breaks in the distribution of immersion duration scores in the saline session. Cold-pain tolerance remained reasonably stable across sessions in the present study, particularly during the first immersion (Figure 2D-F), suggesting that cold-pain tolerance is a stable individual trait. However, the group structure identified in this study needs to be confirmed in a larger sample.

Conclusions

Cold-water immersions induced complex opioid-mediated effects on nociceptive processing. Systemic or central opioid release inhibited cold-evoked pain during lengthy immersions, and inhibited sensitivity to heat at an unburnt site in the non-immersed hand after the immersions. However, in participants who tolerated medium-length immersions, systemic or central opioid release appeared to *augment* sensitivity to heat after the immersions. This hyperalgesic response may have overshadowed inhibitory effects of local opioid release on sensitivity to heat, which were detected only after brief immersions.

Further investigation of the effects identified in the present research may help to clarify the triggers and source of individual differences in peripheral and central opioid anti-nociceptive mechanisms. This could have implications for developing more effective treatment strategies for pain control at sites of inflammation and injury.

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Table 1

Sequence of procedures

1.	Burn injury over the first dorsal interosseous muscle of each hand
2.	Heat-pain threshold (HPT) measured 30 min later at each burn site and at an untreated site on each hand
3.	Double-blind subcutaneous injection of naloxone or saline into one burn site
4.	HPT measured 5 min later at all four sites
5.	Repeated immersion of the injected hand in cold water
6.	Hand re-warmed for 3-5 min to pre-immersion temperature
7.	HPT measured at both sites in both hands straight after the hand was re-warmed, and again 5 min later
8.	Procedures repeated 4-7 days later with subcutaneous injection of the agent (naloxone or saline) not administered in the first session

Table 2

Skin temperature (^{o}C $\pm$ SEM) before and after the immersions

	Naloxone Session		Saline Session	
Site	Before	After	Before	After
Burnt immersed	31.1 $\pm$ .3	31.2 $\pm$ .3	31.3 $\pm$ .4	31.2 $\pm$ .3
Burnt unimmersed	31.5 $\pm$ .3	31.4 $\pm$ .3	31.1 $\pm$ .3	31.3 $\pm$ .4
Unburnt immersed	30.9 $\pm$ .3	30.7 $\pm$ .3	30.6 $\pm$ .3	30.4 $\pm$ .2
Unburnt unimmersed	30.9 $\pm$ .3	30.7 $\pm$ .4	30.9 $\pm$ .4	31.0 $\pm$ .4

Table 3

Sex, age, and mean heat pain thresholds (HPT) in burnt and unburnt skin before the cold-water immersions, in groups differing in cold-pain tolerance

	Pain-Intolerant	Mid-Range	Pain-Tolerant
Males (N)	3	8	6
Females (N)	4	8	3
Age (years) $\pm$ SEM	19.9 $\pm$ 1.3	21.6 $\pm$ 1.2	20.7 $\pm$ 2.4
HPT burnt skin (^{o}C) $\pm$ SEM	40.6 $\pm$ 0.8	40.9 $\pm$ 0.5	41.7 $\pm$ 0.7
HPT unburnt skin (^{o}C) $\pm$ SEM	42.0 $\pm$ 1.0	42.2 $\pm$ 0.7	43.5 $\pm$ 0.9

Note: none of the differences among groups was statistically significant.

Figure captions

Figure 1. Mean immersion duration (in s) in all 32 participants during the saline session plotted against mean immersion duration during the naloxone session (Panel A), and plotted against the difference in immersion duration during the two sessions (Panel B). Note that the mean immersion duration was 180 s during both sessions in three of the participants. The dotted lines in Panel B represent the cut-off points for the pain-intolerant group (mean duration of immersion in the saline session less than 35 s), the mid-range group (range 57-128 s), and the pain-tolerant group (range 142-180 s).

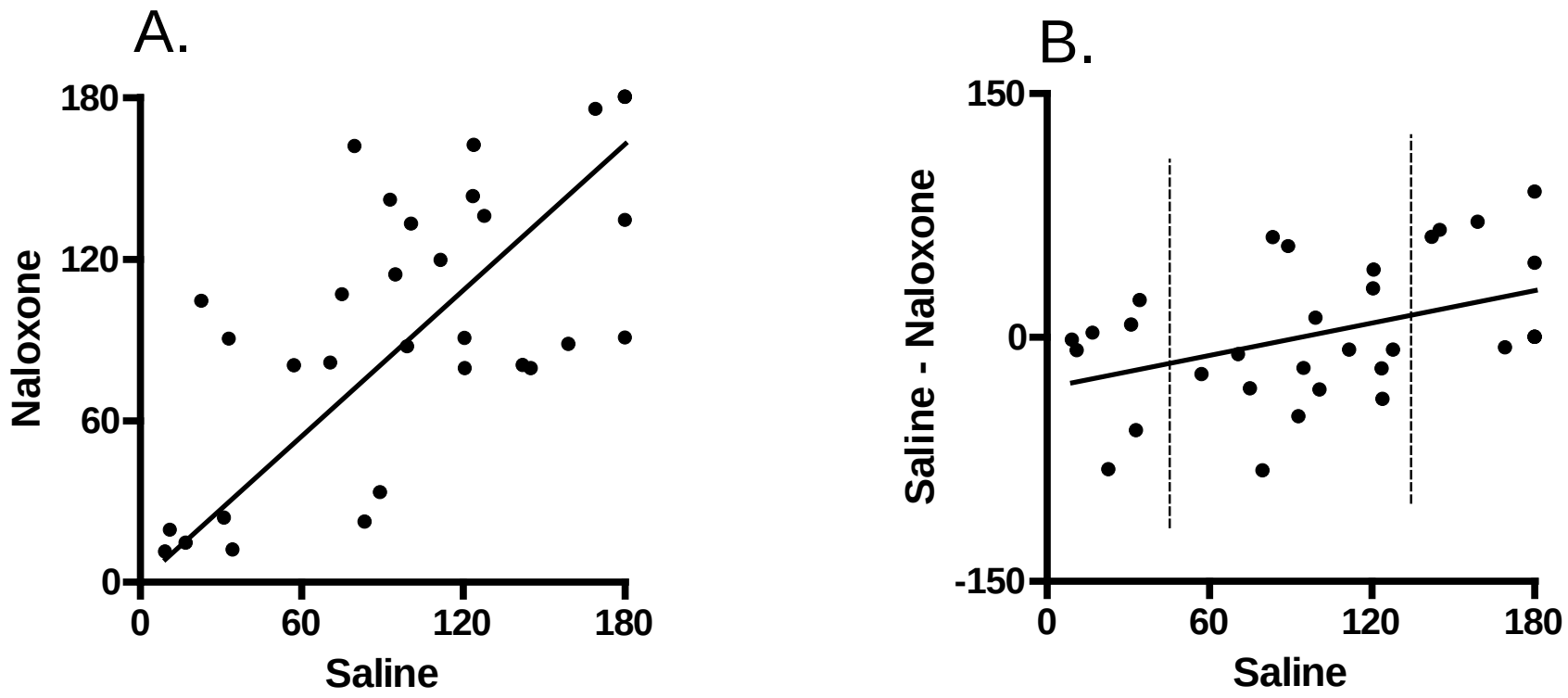
Figure 2. Pain threshold (Panels A-C), pain tolerance (Panels D-F), and pain ratings (Panels G-I) during the first six trials of cold-water immersion for the saline (open symbols, dotted lines) and naloxone (closed symbols, unbroken lines) sessions. In the pain-tolerant group, pain started sooner, tolerance was lower, and pain ratings were greater during certain trials of the naloxone session than the saline session (* $p < 0.05$). Error bars show ± 1 *SEM*.

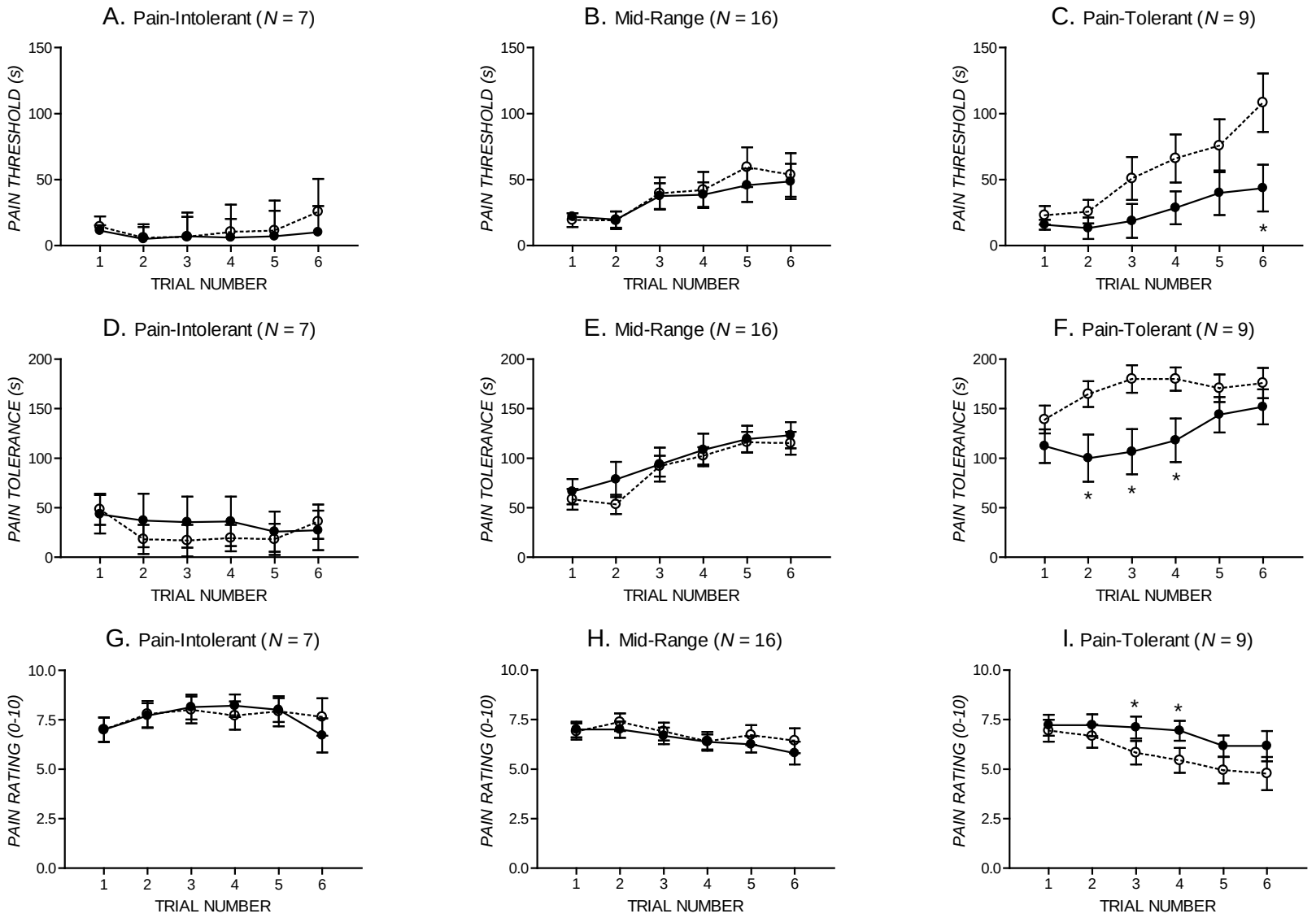
Figure 3. Mean heat-pain thresholds (in degrees C) in the saline (open symbols, dotted lines) and naloxone (closed symbols, unbroken lines) sessions at the burnt and unburnt sites on the immersed and non-immersed hands (Panels A-D). The HPT increased from pre-immersion levels at the burnt site in the immersed hand (* $p < 0.01$) but not at other sites. However, the HPT sometimes differed between consecutive measurement points (# $p < 0.05$). Despite the increase in the HPT at the burnt site after the immersions, the HPT remained lower at burnt (A,B) than unburnt sites (C,D) for the remainder of the experiment ($p < .01$ at all sites at every measurement point). Error bars show ± 1 *SEM* ($n = 32$).

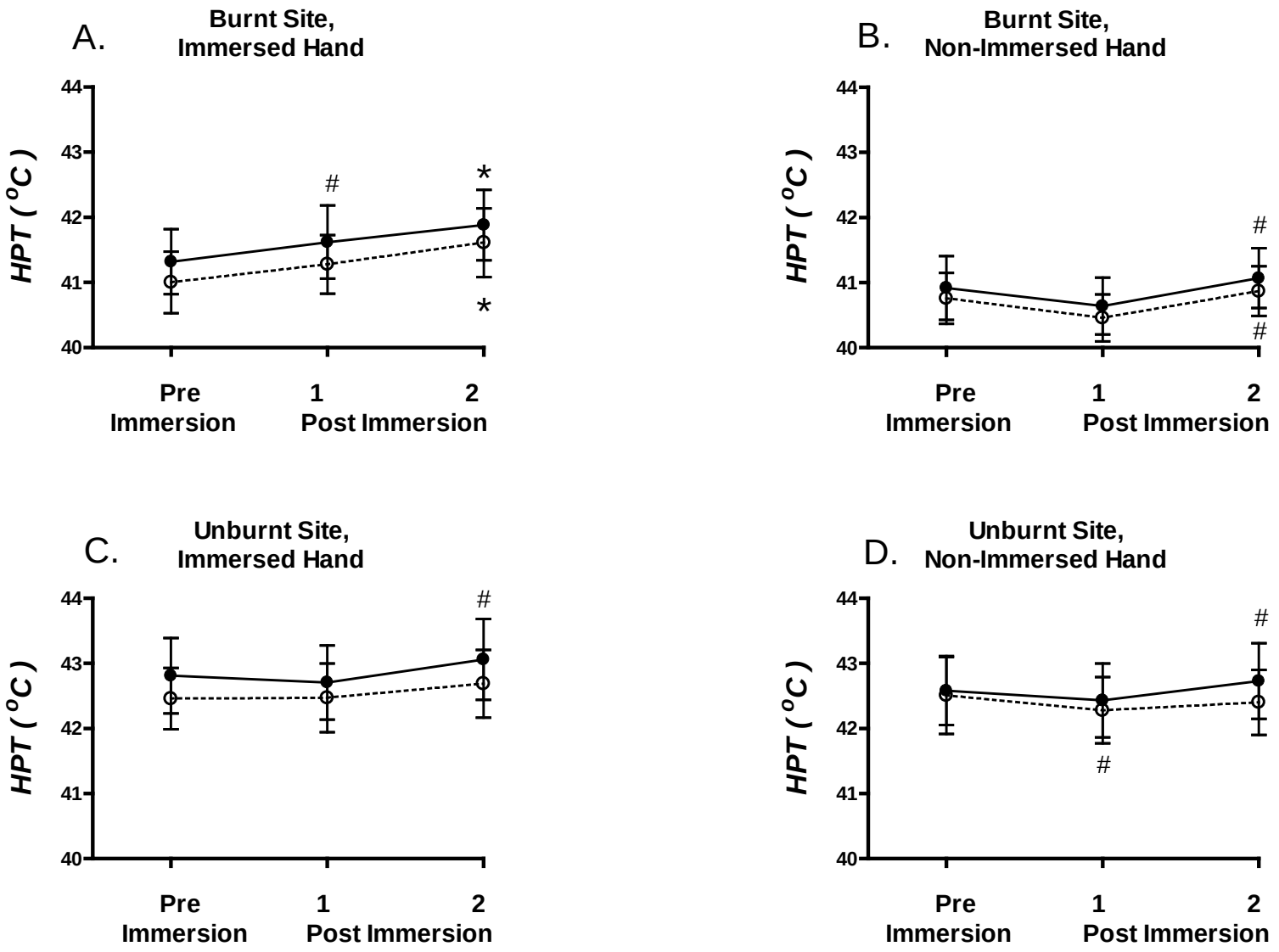
Figure 4. Mean change in heat-pain threshold (in degrees C) from pre-immersion to the second post-immersion measure at unburnt sites in the immersed and non-immersed hands in the saline (open bars) and naloxone sessions (filled bars) in pain-tolerant (Panel A), mid-range (Panel B) and pain-intolerant participants (Panel C). A positive score indicates thermal

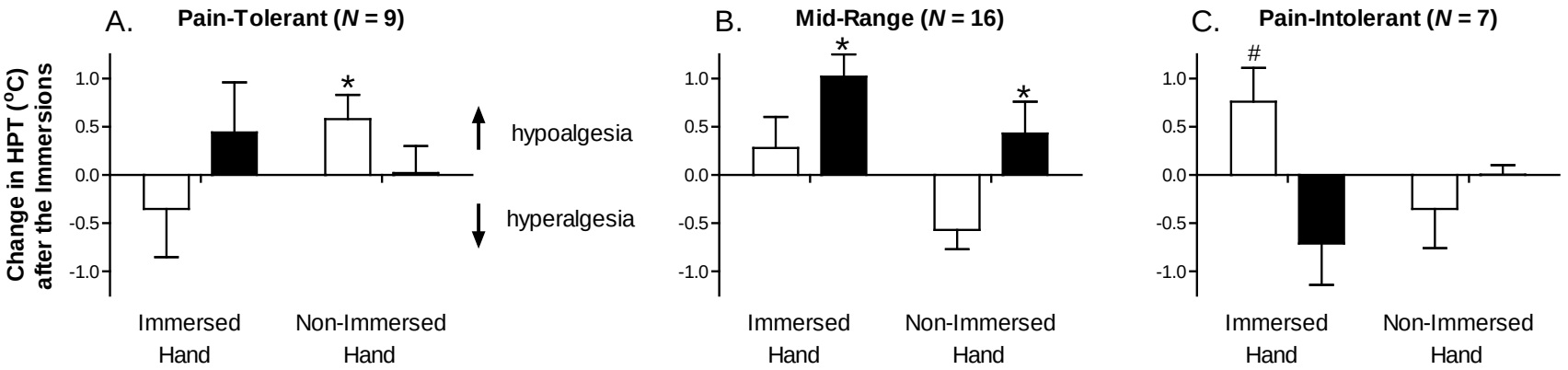
hypoalgesia whereas a negative score indicates thermal hyperalgesia. Naloxone blocked thermal hypoalgesia in the non-immersed hand of pain-tolerant participants (* difference between the saline and naloxone sessions, $p < .05$, Wilcoxon matched-pairs signed ranks two-tailed test), but appeared to mediate thermal hypoalgesia in participants who were neither pain-tolerant nor intolerant (* $p < .05$, Wilcoxon two-tailed test). In pain-intolerant participants, naloxone blocked thermal hypoalgesia in the immersed hand but not the non-immersed hand, consistent with local opioid analgesia (# $p < .05$, Wilcoxon one-tailed test).

Mean Tolerance (s)




A. Pain-Intolerant (N = 7)
B. Mid-Range (N = 16)
C. Pain-Tolerant (N = 9)
D. Pain-Intolerant (N = 7)
E. Mid-Range (N = 16)
F. Pain-Tolerant (N = 9)
G. Pain-Intolerant (N = 7)
H. Mid-Range (N = 16)
I. Pain-Tolerant (N = 9)
PAIN THRESHOLD (s)
PAIN TOLERANCE (s)
PAIN RATING (0-10)
TRIAL NUMBER


A.
Burnt Site,
Immersed Hand
B.
Burnt Site,
Non-Immersed Hand
C.
Unburnt Site,
Immersed Hand
D.
Unburnt Site,
Non-Immersed Hand
HPT (°C)
44
43
42
41
40
#
*
Pre
Immersion
1
2
Post Immersion


A.
Pain-Tolerant (N = 9)
B.
Mid-Range (N = 16)
C.
Pain-Intolerant (N = 7)
Change in HPT (°C) after the Immersions
1.0
0.5
0.0
-0.5
-1.0
hypoalgesia
hyperalgesia
*
#
Immersed Hand
Non-Immersed Hand